GFB-1 DIV1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Not yet assigned		
Group	:	Not yet assigned		
Applicants	:	Jens Kossman et al.		
Application No.:		Not yet assigned;	Confirmation No.:	Not yet assigned
Filed	:	Concurrently herewith		
For	:	DNA SEQUENCES CODING FOR ENZYMES CAPABLE OF FACILITATING THE SYNTHESIS OF LINEAR α-1,4 GLUCANS IN PLANTS, FUNGI AND MICROORGANISMS		

New York, New York
April 26, 2001

Honorable Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examining this application, kindly amend the application as follows:

IN THE SPECIFICATION

Please add the following paragraph as the first sentence of the specification directly following the title:

This application is a divisional of United States Application No. 08/737,752, filed February 27, 1997, which is a 371 of PCT/EP95/01893 filed May 18, 1995.

Replace the paragraph starting on page 30, line 5 with the following paragraph:

The plasmid pNB2 of the invention was deposited at Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Germany, on May 6, 1994 according to the provisions of the Budapest Treaty under deposit no. DSM 9196.

Replace the paragraph starting on page 36, line 26 with the following paragraph:

The resulting fragment contains the coding region for amylosucrase except for the nucleotides coding for the 16 N-terminal amino acids. These amino acids comprise the sequences that are necessary for the secretion of the enzyme from the cell. Furthermore, this PCR fragment contains 88 bp of the 3' untranslated region. By way of the primers used NcoI restriction sites were introduced into both ends of the fragment.

Replace Sequence Listing pages 38-45 with substitute Sequence Listing pages 38-45 submitted herewith.

IN THE CLAIMS

On page 46, before claim 1, add the words --We claim:--.

Cancel claims 1, 3-7 and 9-18.

Amend claim 2 as follows*:

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* An "Appendix of Amendments" is enclosed herewith showing the amendments to the paragraphs on pages 30 and 36 as well as the amendments to claim 2. In the Appendix, the additions are underscored and the deletions are bracketed.

2. (Amended) A DNA molecule coding for a protein having the enzymatic activity of an amylosucrase, obtainable by a process comprising the following steps:

(a) preparing a genomic or a cDNA library;

(b) transforming a suitable host cell with the library constructed according to (a);

(c) subjecting the transformed cells to iodine vapor in the presence of sucrose;

(d) identifying the cells that are stained blue;

(e) isolating and cultivating the cells identified in step (d);

(f) isolating the genomic DNA insert or the cDNA insert from the transformed cell; and

(g) verifying that the protein encoded by the isolated genomic or cDNA molecule has amylosucrase activity.

Add claims 22-45 as follows:

22. (Added) A method of isolating a DNA molecule coding for a protein having the enzymatic activity of an amylosucrase comprising the following steps:

(a) preparing a genomic or a cDNA library;

(b) transforming a suitable host cell with the library constructed according to (a);

(c) subjecting the transformed cells to iodine vapor in the presence of sucrose;

(d) identifying the cells that are stained blue;

(e) isolating and cultivating the cells identified in step (d);

(f) isolating the genomic DNA insert or the cDNA insert from the transformed cell; and

(g) verifying that the protein encoded by the isolated genomic or cDNA molecule has amylosucrase activity.

23. (Added) A method for determining whether a nucleic acid molecule encodes a protein having amylosucrase activity comprising:

(a) introducing a nucleic acid molecule into a host cell;

(b) subjecting the host cell to iodine vapor in the presence of sucrose;

(c) observing whether the host cell stained blue;

(d) isolating the nucleic acid molecule from the cell; and

(e) verifying that the protein expressed from the nucleic acid molecule has amylosucrase activity.

24. (Added) A host cell comprising the DNA molecule according to claim 2.

25. (Added) The host cell of claim 24 wherein said host cell is a plant cell.

26. (Added) The host cell of claim 24 wherein said host cell is a fungal cell.

27. (Added) A microorganism comprising the DNA molecule according to claim 2.

28. (Added) A transgenic plant comprising the DNA molecule according to claim 2.

29. (Added) The plant according to claim 28 wherein the plant is a crop plant.

30. (Added) The plant according to claim 28 selected from the group consisting of maize, rice, wheat, barley, sugar beet, sugar cane, tobacco, tomato, and potato plant.

31. (Added) A vector comprising the DNA molecule according to claim 2.

32. (Added) The vector according to claim 31 wherein said DNA molecule is operably linked to promoter sequences.

33. (Added) A process for the production of linear α-1,4 glucans, fructose and/or fructose syrup comprising the steps of:

(a) culturing the host cell according to claim 24 or the microorganism according to claim 27, wherein the host cell or the microorganism secretes the amylosucrase into a culture medium comprising sucrose under conditions allowing expression and secretion of the amylosucrase; and

(b) recovering the produced α-1,4 glucans and/or fructose from the culture medium.

34. (Added) The process according to claim 33, wherein the host cell is immobilized.

35. (Added) A process for the production of linear α-1,4 glucans comprising the steps of:

(a) producing an expression cassette comprising the following DNA sequences:

(i) a promoter that is active in plants and ensures formation of an RNA in the respective target tissue or target cells;

(ii) the DNA molecule according to claim 2 encoding a protein having the enzymatic activity of an amylosucrase which is fused to the promoter in sense orientation; and

(iii) a signal sequence functional in plants for transcription termination and polyadenylation of an RNA molecule.

(b) transferring the expression cassette into a plant cell;

(c) regenerating a transgenic plant from the transformed plant cell; and

(d) isolating the linear α-1,4 glucans synthesized in the plant from the plant.

36. (Added) The process according to claim 35, wherein the expression cassette contains a nucleotide sequence encoding a transit peptide which ensures transport of the protein having the enzymatic activity of an amylosucrase to a vacuole or to an apoplast.

37. (Added) The process according to claim 35, wherein the DNA sequence as indicated in (ii) which codes for a protein having the enzymatic activity of an amylosucrase does not contain a signal sequence effecting secretion to a apoplast.

38. (Added) The process according to claim 35, wherein the promoter defined in (i) ensures the expression of amylosucrase in sucrose storage organs of the plant.

39. (Added) A linear α-1,4 glucan obtainable by the process of any one of claims 35 to 38.

40. (Added) A process for the production of linear α-1,4 glucans, fructose and/or fructose syrup in vitro comprising the steps of:

(a) contacting a solution comprising sucrose with a protein according to claim 8 under conditions allowing the conversion of sucrose to α-1,4 glucans and fructose by the amylosucrase; and

(b) recovering the produced α-1,4 glucans and/or fructose from the solution.

41. (Added) The process according to claim 40, wherein the protein is immobilized.

42. (Added) A protein having the activity of an amylosucrase encoded by the amylosucrase coding region in the DNA insert of plasmid pNB2 from Neisseria bacteria having deposit number Deutsche Sammlung von Mikroorganismen No. 9196, by a DNA sequence that hybridizes to that coding region, or by a degenerate DNA sequence of any of the aforementioned sequences encoding a protein having the activity of amylosucrase.

43. (Added) A protein having the activity of an amylosucrase encoded by a DNA sequence that begins at the initiating codon in the DNA insert of plasmid pNB2 from Neisseria bacteria having deposit number Deutsche Sammlung von Mikroorganismen No. 9196 as indicated in Seq ID No. 1 and ends at the first stop codon located in frame downstream of the initiating codon in that DNA insert.

44. (Added) A fusion protein comprising a protein having the activity of an amylosucrase, wherein the protein having the activity of an amylosucrase is encoded by the amylosucrase coding region in the DNA insert of plasmid pNB2 from Neisseria bacteria having deposit number Deutsche Sammlung von Mikroorganismen No. 9196, by a DNA sequence that hybridizes to that coding region, or by a degenerate DNA sequence of any of the aforementioned sequences encoding a protein having the activity of amylosucrase.

45. (Added) A fusion protein comprising a protein having amylosucrase activity, wherein the protein having amylosucrase activity is encoded by a DNA sequence that begins at the initiating codon in the DNA insert of plasmid pNB2 from Neisseria bacteria having deposit number Deutsche Sammlung von Mikroorganismen No. 9196 as indicated in Seq ID No. 1 and ends at the first stop codon located in frame downstream of the initiating codon in that DNA insert.

REMARKS

The Specification

Applicants have amended the specification to make reference to a priority claim to U.S. Application No. 08/737,752 under 35 U.S.C. §121.

Applicants have amended the paragraph starting on page 30, line 5 solely to correct the spelling of "deposit."

Applicants have amended the paragraph starting on page 36, line 26 solely to correct the spelling of "introduced."

Pursuant to 37 C.F.R. § 1.825(a), applicants have amended the specification by submitting substitute Sequence Listing pages 38-45. Also filed concurrently herewith is a statement under 37 C.F.R. § 1.821(e) to authorize the use of the computer readable form submitted in parent application U.S. Application No. 08/737,752 ("the '752 application") on October 13, 1998, in this application. Applicants submit herewith a statement verifying that the attached substitute Sequence Listing and the last-filed computer readable form submission in the '752 application (submitted October 13, 1998) are the same and do not include new matter. Applicants request that the Patent Office use the last-filed computer readable form submission of the '752 application in the present application.

The Claims

Applicants have canceled claims 1, 3-7, and 9-18 without prejudice.

Applicants have amended claim 2 to improve its form and also to include the step of verifying that the protein expressed from the genomic or cDNA molecule has amylosucrase activity. Support for amended claim 2 may be found *inter alia*, at page 5, lines 11-24, at page 7, line 28 to page 8, line 2, and at page 9, lines 1-21.

Applicants have added claim 22 directed to a method of isolating a DNA molecule coding for a protein having the amylosucrase activity. Applicants have also added claim 23 directed to a method of determining whether a nucleic acid molecule encodes a

protein having amylosucrase activity. Support for claims 22 and 23 may be found, *inter alia*, at page 5, lines 11-24, at page 7, line 28 to page 8, line 2, and at page 9, lines 1-21.

Applicants have added claims 24-27 directed toward host cells and microorganisms comprising the DNA molecule according to claim 2. Support for claims 24-27 may be found, *inter alia*, at page 9, lines 23-32 and at page 10, lines 32-34.

Applicants have added claims 28-30 directed to transgenic plants comprising the DNA molecule according to claim 2. Support for claims 28-30 may be found, *inter alia*, at page 15, lines 24-35.

Applicants have added claims 31 and 32 directed toward vectors comprising the DNA molecule according to claim 2. Support for claims 31 and 32 may be found, *inter alia*, at page 10, lines 1-31.

Applicants have added claims 33 and 34 depending therefrom directed to a process for the production of linear α-1,4 glucans, fructose and/or fructose syrup. Support for these claims may be found, *inter alia*, at page 21, line 22 to page 28, line 14.

Applicants have added claims 35-39 directed to processes for the production of linear α-1,4 glucans and the linear α-1,4 glucans obtainable by the processes. Support for these claims can be found, *inter alia*, at page 12, line 37 to page 13, line 11; at page 14, lines 1-35; page 16, lines 10-16

Applicants have added claims 40 and 41 depending therefrom directed to a process for the production of linear α-1,4 glucans, fructose and/or fructose syrup in vitro. Support for these claims may be found, *inter alia*, at page 24, line 14 to page 26, line 18 and at page 28, line 14 to page 29, line 25.

Applicants have added claims 42 and 43 directed to proteins having the activity of an amylosucrase. Support for these claims may be found, *inter alia*, at page 7, lines 12-27;

at page 30, lines 5-8; at page 31, lines 3-13; at page 32, line 14 to page 35, line 22 (Examples 3 and 4); and in Figure 2.

Applicants have added claims 44 and 45 directed to fusion proteins comprising a protein having the activity of an amylosucrase. Support for these claims may be found, inter alia, at page 24, line 32 to page 25, line 13; at page 30, lines 5-8; and at page 39.

After entry of the amendments and added claims, claims 2, 8 and 19-45 are pending in the application.

No new matter is introduced. Entry of the amendments is requested.

Respectfully submitted,

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Appendix of Amendments

IN THE SPECIFICATION

The paragraph starting on page 30, line 5:

The plasmid pNB2 of the invention was deposited at Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Germany, on May 6, 1994 according to the provisions of the Budapest Treaty under [depost] deposit no. DSM 9196.

The paragraph starting on page 36, line 26:

The resulting fragment contains the coding region for amylosucrase except for the nucleotides coding for the 16 N-terminal amino acids. These amino acids comprise the sequences that are necessary for the secretion of the enzyme from the cell. Futhermore, this PCR fragment contains 88 bp of the 3' untranslated region. By way of the primers used NcoI restriction sites were [intoduced] introduced into both ends of the fragment.

IN THE CLAIMS

2. A DNA [sequence] molecule coding for a protein having the enzymatic activity of an amylosucrase, obtainable by a process comprising the following steps:

(a) preparing a genomic or a cDNA library [on the basis of the genomic DNA or the mRNA of cells of an organism];

(b) transforming a suitable host cell with the library constructed according to (a);

(c) subjecting the transformed cells to iodine vapor in the presence of sucrose;

(d) identifying the cells that are stained blue;

(e) isolating and cultivating the cells identified in step (d); [and]

(f) isolating the genomic DNA insert or the cDNA insert from the transformed cell[.] ; and

(g) verifying that the protein encoded by the isolated genomic or cDNA molecule has amylosucrase activity.